A DISSERTATION ON

CLINICAL PROFILE OF HYPOCHROMIC MICROCYTIC ANEMIA

M.D (BRANCH VII)

PAEDIATRIC MEDICINE

MARCH 2010

THE TAMILNADU

DR.MGR.MEDICAL UNIVERSITY

CHENNAI, TAMILNADU

CERTIFICATE

This is to certify that the dissertation titled **"A DISSERTATION ON CLINICAL PROFILE OF HYPOCHROMIC MICROCYTIC ANEMIA"** submitted by **Dr.P. VENKATESH** to the Faculty of pediatrics, The Tamilnadu M.G.R.Medical University,Chennai in partial fulfillment of the requirement for the award of M.D.Degree (Pediatrics) is a bonafide research work carried out by her under our direct supervision and guidance.

Dr.P.Amutha Rajeshwari,M.D.,DCH.,

Professor and Head of the Department,
Institute of Child Health & Research Centre,
Madurai Medical College,
Madurai.

DECLARATION

I **Dr.P. VENKATESH**, solemnly declare that the dissertation titled "**A DISSERTATION ON CLINICAL PROFILE OF HYPOCHROMIC MICROCYTIC ANEMIA"** has been prepared by me.

This is submitted to the **Tamilnadu Dr.M.G.R.Medical University**, Chennai in partial fulfillment of the rules and regulations for the M.D.Degree Examination in Paediatrics.

Place: Madurai

Date: **Dr. P. VENKATESH**

ACKNOWLEDGEMENT

It is with immense pleasure and privilege that I express my heartful gratitude, admiration and sincere thanks to **Prof.Dr.P.AmuthaRajeshwari,** Professor and Head of the Department of Pediatrics, for her guidance and support during this study.

I am greatly indebted to my guide and teacher, **Prof.Dr.T. Nagarajan,** Professor of Paediatrics, for his supervision, guidance and encouragement while undertaking this study.

I express my sincere thanks and gratitude to **Prof.Dr.M.L.Vasanthakumari** for her support, guidance and encouragement while carrying out the study.

I would like to thank **Prof. Dr. G. Mathevan, Prof. Dr. S. Rajasekaran, Prof. Dr.Chitra Ayyappan, Prof. Dr. R.A.S. Sankara subramanian,** who guided me to a great extent. I also thank all the members of the Dissertation committee for their valuable suggestions.

I wish to express my sincere thanks to **Prof. Dr.S.Shanmugasundaram**, for his constant supervision, guidance and encouragement.

I gratefully acknowledge the help and guidance received from **Asst.Professor Dr.M.S.Rajarajeswaran and other Asst.professors** for their constant support and suggestions during this study.

I also express my gratitude to all my fellow postgraduates for their kind cooperation in carrying out this study and for their critical analysis and also I thank the **healthworker Mr.Prabhu** for his cooperation.

I thank our **DEAN and the members of Ethics committee**, Government Rajaji Hospital and Madurai Medical College, Madurai for permitting me to perform this study.

I thank all the parents and children who have ungrudgingly lent themselves to undergo this study without whom this study wouldnot have seen the light of the day.

CONTENTS

INTRODUCTION

Children of today are citizens of tomorrow and upon them depend the weal and welfare of the community. In a country like India, children fall an easy prey to anemia as majority of them remain ill-fed, ill-clothed and undernourished due to poverty and ignorance. If not detected at the earliest point of time, this disease which is draconian will spread it's tentacles so widely as to impair or endanger the very physical condition of the children.

Among the haematological disorders, **anemia is the commonest entity of multiple aetiologies.** The meaning of Greek word "anemia" is without blood.

It is a major world health problem and is an important cause of morbidity and mortality much of which can be preventable. Man has only partially adapted to the rapid growth in human population and environmental changes and anemia is one of the effects of these factors. Thus still the most common cause of anemia in children is related to nutritional deficiency especially iron deficiency. Initially man depend mostly on animal food and as he learned agricultural practices, the contribution of animal food

reduced drastically to less than 5%. This profoundly affected the bioavailable iron and folate resulting in nutritional deficiency anemia.

Anemia is defined as the reduction of RBC volume or hemoglobin concentration below the range of values for a particular age and sex.

The detection and diagnosis of anemia are frequently the focus of attention in the care of patients because accurate quantification and rational analysis of problem is must.

Anemia is not a diagnosis in itself like fever but merely is an objective sign of the presence of disease. The correct diagnostic terminology for a child with anemia requires the detection of etiology, pathology and pathogenesis of the anemia.

A systematic approach through proper history, physical examination and relevant investigations are very essential to diagnose the various causes of anemia.

This study have evaluated the clinical profile of hypochromic microcytic anemia among the children. In the recent era various newer diagnostic modalities are available to findout the

cause of anemia. But most of them are out of reach of the hands of the children of lower socioeconomic status. Nutritional deficiency anemia particularly iron deficiency anemia is the most common type of anemia among these children.

In countries like India iron deficiency anemia coexists with other types of anemia in almost every child with anemia. This iron deficiency masks the underlying diseases especially thalassemia trait which cannot be diagnosed even by higher investigations like Hb electrophoresis unless the iron deficiency is corrected prior to the electrophoresis. Though thalassemia trait cases can lead a normal life, unless they are identified and genetic counselling given,they will transmit the gene to next generation leading to future thalassemia major cases which is a major burden to the family as well as the society, which is a preventable one.

Beta thalassemia is the most common single gene disorder in our country. Beta thalassemia major is a homozygous severe transfusion dependent condition with most of the children dying in childhood. Beta thalassemia trait is a heterozygous genetic disorder

having normal growth and development and individual may occasionally have mild anemia

To prevent the birth of a homozygous child, the best option is identification of carrier state, which is a valid alternative to managing thalassemic mojor children. This disease is a prime example in which prevention has primary importance and priority over treatment, thus prenatal diagnosis has been the main stay of most control programmes. As with any autosomal recessive disorder, the incidence of the homozygote can be decreased, by detection of a carrier state.

With the consideration of all the above problems started this study is undertaken to formulate an approach to hypochromic microcytic anemia in children which is easy, feasible, cost effective and can be followed in resource poor settings. Even it is the ideal approach to all cases of hypochromic microcytic anemia inorder to overcome the above problems and to find out the exact cause of hypochromic microcytic RBC’s.

REVIEW OF LITERATURE

Historical aspects:

The basic concepts of hematology came largely from internal medicine and from the experimental sciences. Every paediatrician, particularly haematologists, must remember the names such as Ehrlich, Metchnikoff, Landsteiner, Chauffard, Downey, Minot, Castle, Whipple and Wintrobe who have pioneered in hematology. Traditionally, the history of anemia began in 1889 with Von Jaksh's report on the condition that bears his name which he designated pseudo leucaemica infantum [53,54]

In 1919,Winifred Ashby measured the normal life span of RBC's of adult using the differential agglutination[60]

More lasting and far more important was the contribution made to paediatric hematology by Thomas.B.Cooley in 1925 when he salvaged a distinct entity now known as thalassemia [55]

In 1927,Heinrich Baar wrote, "The hemoglobin content suffers more than the RBC count" in anemia.

George Guest conducted meticulous studies between 1932 and 1942 with regards to Hb levels, RBC, Packed cell volumes of a

large group of infants and young children and showed convincingly that a fall in the mean corpuscular hemoglobin (MCV) in the presence of seemingly adequate Hb levels could be normalised by administration of Iron and was the first person to observe iron deficiency anemia of infancy.

In 1944, Blackfan and diamond's Atlas of the blood in children was published, which used wintrobe's classification and described the principal anemia of infancy under the title "iron deficiency anemia" [59]

In 1959, Ingram and Stretton postulated two classes of thalassemia , α and β thalassemia, corresponding to α and β chain variants of the Hemoglobinopathies.

EPIDEMIOLOGY

Anemia is a global health problem and hypochromic microcytic anemias due to iron deficiency is the most common nutritional disease prevalent in developing countries.

80 % of children in developing countries and 20% of children in developed countries are anemic according to MARTIN PL et al[8] .Prevalence of anemia in children is 51% and girls had a higher prevalence of anemias in another study[9] Nearly the half of well nourished children are anemic (47.6%[9]).

The NFHS survey II (1998-99) estimated the prevalence of anemia as 74% among children aged 6 months-3 years and severe anemia as 1.3%.(Hb <7 gm/dl).[10]

Kapoor D et al and padmanaban A et al estimated the incidence of anemia in children at 64% and 37.9-45.1% respectively[11,12.]

El hazimi et al [13] estimated the prevalence of anemia in children as 24.8%.

In infants and children the most common cause of anemia is micro cytic hypo chromic anemia,accounting for 55.4 – 64% of

total anemias[9,14,15]. The prevalence of iron deficiency anemia in the United States ranges from 3 to 10 percent and may be as high as 30 percent in low-income populations

Azmath manzoor et al & Osk et al put the prevalence of iron deficiency anemia among total anemias at 62.9 % and 10% respectively[12,13].

D Viswanath et al and Alukh et al revealed that 89% and 64% of children with Microcytic hypochromic anemia had Iron Deficiency anemia.[15,16].

Prevalence of thalassemia is among communities of Punjabis,sindhis,gujaratis and parsis. India has an estimated four crore thalassemia carriers and 10,000 thalassemia major children are born in India every year.

Prevalence of thalassemia in general population varies from 2.7% in Mumbai,5.5% in Delhi,and 10.4% in kolkata. [17.] Incidence of Thalassemia minor and Thalassemia major among anemic children varies from 14.34% to 4.7%.

A recent study of Saradha Sidhu (1996) on prevalence of anemia among scheduled caste preschool children, reported a prevalence rate of 55%. [57]

As per ICMR study (V.P Choundhry et al in 1995), it has been estimated that over 60% of rural children between 1 to 3 years have anemia and 40% of children between 3 to 6 years are anemic and iron deficiency was proved to be the commonest cause. [56]

A prevalence of 60% was reported in children less than 4 years in a study conducted in 331 Filipino children between 9 months to 7 years by Marzan et al. a strong association was found between anemia and nutritional status.

ERYTHROPOEISIS

The primitive erythropoeisis arises in the blood islands of the extraembryonic yolksac and shifts to the splancno-pleural/aorta-gonad mesonephros of the developing embryo. Production then shifts to liver and spleen by about the seventh month of gestation.Bone marrow replaces the liver and spleen as the principal site of production after birth.

In new born, hematopoeitic tissue fills all cavities within the bones and with increasing age it becomes localized to cavities of upper shaft of femur,humerus the pelvis ,spine,skull and bones of thorax.

DEVELOPMENT OF BLOOD CELLS

Blood cells develop from a small population of toti-potent hematopoeitic stem cells which give rise to hematopoeitic cell series.

Red cells are produced by proliferation and differentiation of precursors whose dominant representatives in the bone marrow are the erythroblasts.

During the course of differentiation, the size of erythroblasts progressively decreases and the character of nucleus and cytoplasm changes as the cells proceed toward the point where proliferative capacity is lost and hemoglobin becomes the dominant protein in the cytoplasm.

The PRO-ERTHROBLAST is the least mature of the morphologically identifiable members of the erythroid series. It has a diameter of 14-20 microns and a basically round outline with

minor peripheral protuberances. Nucleus is large, round with fine chromatin, several nucleoli and basophilic cytoplasm.

Proerythroblast gives rise to basophilic erythroblast, a round cell with diameter of 12-16 microns and more basophilic cytoplasm than the pro-erythroblast. The nucleus occupies a relatively large proportion of the cell having coarser and more basophilic chromatin strands.

Next stage is represented by the polychromatic erythroblast, a round cell measuring 12-14 microns. The characteristic polychromatic appearance of the cytoplasm is derived from the mixture of the basophilic RNA and acidophilic hemoglobin. Nuclear chromatin is in coarse, deeply basophilic clumps. Proliferative activity ceases at this stage. Hemoglobin which is found in the cytoplasm possibly gains entrance into the nucleus through pores in the nuclear membrane.,and after reaching a critical concentration in the nucleus (20gm/dl) reacts with nucleohistones, thereby bringing about chromosomal inactivation and nuclear condensation.[1-3]

Orthochromatic erythroblasts are the last cells of nucleated red cell series. They measure 8-12 microns with a small and pyknotic nucleus with a homogenous blue-black appearance. Active hemoglobin synthesis occurs in the cytoplasm ,which contains mitochondria and ribosomes.

Nucleus is extruded from the orthochromatic erythroblast and reticulocytes are formed with diffuse basophilic hue of cytoplasm. Reticulocytes have the same biconcave discoid shape as mature red cells with slightly greater volume and diameter.

Reticulocytes enter the circulation and lose their mitochondria and ribosomes over a course of few days and evolve into mature RBCs.

STRUCTURE AND METABOLISM OF RED CELLS

Erythrocytes are biconcave disc shaped cells with a mean diameter of 7.2-7.9 microns. They normally lack nuclei and cytoplasmic structures such as lysosomes, endoplasmic reticulum and mitochondria. They exist in large blood vessels as biconcave discs, but change their shape to parachute like conformation in small capillaries with diameter less than that of red cell.

The membrane of the RBC is a highly deformable, but non-expansile or contractile structure. It's integrity is firmly maintained by the attachment of it's inner surface to a lattice like structure of specialized cytoskeletal proteins which supports the membrane and dictates the shape of the erythrocyte.

The major protein component of the membrane is **band 3** protein which spans the full width of membrane and encases channels which facilitates transport of glucose and anions.

Another important transmembrane protein is **glycophorin** .

The exterior surface of this molecule is heavily substituted with sugars containing sialic acid which contributes to the negative charge of the outer surface of red cell at physiological pH.

Glycolipids in the outer leaflet of membrane contain specific oligosaccharide sequences which constitute the ABO blood group substances. Phospholipid and cholesterol are the dominant lipid components of the matrix of the membrane.

The most important constituent of the cytoskeleton is the protein SPECTRIN. Inter twined spectrin molecules are linked together by a specific protein and action to form a lattice like

network which is attached to the inner surface of the membrane. This network is a resilient structure which normally causes red cells to resume the biconcave disc form after forces causing distortion are removed.

Specific sites on spectrin serves as points of attachment to molecules that protrude from the membrane (band 4.1 protein) and to the protein ankyrin which links the spectrin to the internal pole of the band 3 protein.

Hemoglobin

Hemoglobin molecules consist of two pairs of polypeptide globin chains. Each globin chain bears a haem group whose central iron atom is the site at which oxygen attaches to hemoglobin.

The type of globin chain synthesized by erythroid precursors undergoes progressive change with time after conception .

The embryonic (Hb Gower 1 & 2), predominate until third month of gestation after which fetal Hb becomes the major form.
HbF is the primary hemoglobin found in the fetus.Fetal hemoglobin consists of two ά and γ globin chains. It has a higher affinity for oxygen than adult hemoglobin, thus increasing the efficiency of

oxygen transfer to the fetus. The relative quantities of HbF rapidly decrease to trace levels by the age of six to 12 months and are ultimately replaced by the adult forms, HbA and HbA2.

Adult Hemoglobin consists of two ά and two β globin chains.Significant amount of adult hemoglobin is synthesized during third trimester of gestation. The proportion of adult Hb progressively increases to 25% of total hemoglobin at birth,and about 97% 12 months later. Another minor form of adult hemoglobin is HbA_2 which is made up of two ά and two δ globin chains.It is present only in trace amounts in the fetus and does not exceed 2% of total hemoglobin in normal adults.

CLASSIFICATION OF ANEMIA [7]

Anemias are classified on the basis of red cell morphology as microcytic hypochromic, normocytic normochromic and macrocytic anemias. The main advantage of classification is it is simple, based on readily available red cell indices and forces the physicians to consider the most important types of curable anemia such as iron deficiency anemia, and anemia due to vitamin B12 and folic acid deficiency.

Such practical considerations has led to the wide acceptance of this classification.

Microcytic hypo chromic anemia.

RBC are smaller in size with exaggerated central pallor. The MCV is less than 75 fl and MCH less than 24 pg respectively.

Causes of hypochromic microcytic anemia

1) Iron deficiency
2) Thalassemia
3) Variant hemoglobin
4) Anemia of chronic disease
5) Sideroblastic anemia
6) Chronic lead and aluminium toxicity
7) Copper deficiency

Normo cytic normo chromic anemias.

The RBC have normal size and Hemoglobinization in the blood films. MCV, MCH are within normal limits. Causes are red cell membrane defect, enzyme defects, acquired hemolytic anemias due to anti bodies, microangiopathic hemolytic anemias , acute red cell loss, hyper splenism and chronic kidney disease.

Macro cytic anemia

The RBC are larger in size and lack central pallor.

MCV , MCH increased and MCHC is with in normal limits .

Common causes are Vitamin B12 and Folic acid deficiency, Aplastic anemias, Hypothyroidism, Liver disease, Bone marrow infiltration.

DEFINITION OF ANEMIA

There are various definitions for anemia. A functional definition of anemia is a state in which, the circulating red cell mass is insufficient to meet the oxygen requirement of the tissues.

G.C. De Gruchy defined anemia as, a reduction in the concentration of hemoglobin in the peripheral blood below the normal for age and sex of the patient.[58]

Maxwell M. Wintrobe described anemia as decrease in concentration of oxygen carrying substance in a certain volume of blood. [61]

A working or clinically useful definition is reduction in concentration of Hb per unit volume of blood of two standard deviations below the normal for that population, age and sex.[59]

Such a precise definition is essential because the symptoms of anemia are often non specific and can be misinterpreted as symptoms of emotional, cardiovascular or respiratory disorder and the reference values vary for different groups of population.

WHO definition of Anemia: **WHO expert group**[4] proposed that anemia should be considered to exist when Hb is below 11 grams% in children aged 6 months to 6 years and 12 gms % in children aged 6-12 years. Severity of the anemia can be graded as

Mild anemia – 10-11gms[5,]

Moderate anemia – 7-10gms%,

Severe anemia < 7gms %[6]

IRON DEFICIENCY ANEMIA

Anemia in the developing world is most commonly caused by an iron deficiency, which affects up to 50 percent of the population in some countries. Estimates suggest that over one third of the world's population suffers from anemia, mostly iron deficiency anemia.

India continues to be one of the countries with very high prevalence. National Family Health Survey (NFHS-3) reveals the

prevalence of anemia to be 70-80% in children, 70% in pregnant women and 24% in adult men.

Prevalence of anemia in India is high because of low dietary intake, poor availability of iron and chronic blood loss due to hook worm infestation and malaria. While anemia has well known adverse effects on physical and cognitive performance of individuals, the true toll of iron deficiency anemia lies in the ill-effects on maternal and fetal health. Poor nutritional status and anemia in pregnancy have consequences that extend over generations.

The most common cause of Microcytic hypochromic anemia is Iron Deficiency anemia.

For normal iron balance 1 mg of iron must be absorbed from diet everyday.Iron deficiency anemias can result from dietary lack of iron, Impaired absorption, increased requirement and chronic blood loss.

In developing countries helminthic infestations,malaria and malnutrition are also common causal factors.[20]

In developed countries introduction of unmodified cow's milk and defective absorption are also implicated. [21,22,23.]

Three stages are discussed in IDA;

A)First stage characterized by decreased storage of iron without any detected abnormalities.

B)An intermediate stage of latent iron deficiency in which anemia has not occurred yet but iron stores are exhausted.

C)Third stage is overt iron deficiency when there is a decrease in concentration of Hb due to impaired Hb synthesis.

As Iron deficiency progresses morphological changes in rbcs follow development of anemias. The production of hypochromic microcytic rbcs is primarily attributed to delay in the synthesis of Hemoglobin.

During erythropoeisis Hb enters the nucleus and it reaches a critical concentration in the nucleus and reacts with nucleohistones causing nuclear condensation and chromosomal inactivation.

Microcytic cells are produced in iron deficiency because it takes longer to reach the critical hemoglobin concentration and the

generation time is unaffected.Hence more cell divisions occur before nuclear inactivation and the resulting cell is small[1,2,3].

Impaired red cell production occurs in association with chronic diseases like osteomyelitis, lung abscess,rheumatoid arthritis, bacterial endocarditis which closely resembles Iron deficiency anemia.

In these conditions there is some impediment in the transfer of iron from the storage pool to the erythroid precursors. In addition, marrow erythroid progenitors do not proliferate adequately because erythropoeitin levels are inappropriately low for the degree of anemia.

The reduction in renal erthropoeitin generation is attributed to production of IL-1,tumor necrosis factor(TNF) and interferon-γ .

These cytokines also inhibit the release of iron from the storage pool.

The anemia is usually mild and the dominant symptoms are those of the underlying disease. The red blood cells can be normocytic and normochromic or hypochromic and microcytic as in anemia of iron deficiency. Presence of low serum iron and

reduced total iron-binding capacity in association with abundant stored iron in the mononuclear phagocytic cells are the common features.

Thalassemias are the most common single gene disorder in the world and represent a major health burden world wide. Approximately 3% of the worlds population are carriers of a beta thalassaemia mutation [19]. In 1925 Thomas Cooley & Pearl Lee described a form of severe anemia occurring in children of Italian origin which was associated with splenomegaly and characteristic bone changes. [24]

Because all early cases were reported in children of Mediterranean origin, the disease was later termed Thalassemia from the Greek word for sea, “thalassa”.[65] The first case of β Thalassemia in India was reported by Dr.Mukerjee from Calcutta in 1938. [18]

Thalassemias are a large and heterogeneous group of red cell disorders resulting from impaired or absent synthesis of globin chains. The different globin chains are coded for on either chromosome 16 (alpha) or 11 (Beta, delta & gamma chains). Thus,

alpha globin synthesis is reduced in alpha thalassemia and beta globin synthesis in beta thalassemia, alpha and beta synthesis in alpha-beta thalassemia, and so forth. In some contexts it is also useful to sub classify the syndromes according to whether synthesis of the affected globin chain is totally absent (i.e., beta$^{(-)}$ thalassemia) or only partially reduced (i.e., beta $^{(+)}$ thalassemia).

Over 250 million people in the world and around 20 million in India carry the gene for beta thalassemia .One lakh children are born world over every year with the homozygous state for thalassemia, 8000-10,000 children of whom are born in India.[18] Frequency of thalassemia trait varies from 3-17% in different populations.

The thalassemias are all caused by mutations in the globin gene cluster. The defects are numerous,**(**more than 200 different mutations have been described[19]**)** and include deletional or non-deletional mutations. Mutations affect every step in the pathway of globin gene expression; transcriptions, processing of the mRNA precursor, translation of mature mRNA and post translational integrity of the β poly peptide chain. Mutations usually have a

geographic and ethnic distribution. They are inherited in a multitude of genetic combinations which are responsible for the heterogeneous group of clinical syndromes. Beta-thalassemia major, also known as Cooley's anemia or homozygous beta-thalassemia, is a clinically severe disorder due to inheritance of two beta-thalassemia alleles, one on each copy of chromosome[11]. HbF levels are elevated in beta-thalassemia. The diagnosis of homozygous state is usually clinically and by Hb F estimation.

The term beta-thalassemia intermedia is applied to a less severe clinical phenotype in which significant anemia occurs but chronic transfusion therapy is not absolutely required. It is usually due to inheritance of two beta-thalassemia mutations, one mild and one severe or inheritance of two mild mutations.

Thalassemia minor, also known as beta-thalassemia trait or heterozygous beta-thalassemia, is due to the presence of a single beta thalassemia mutation and a normal beta globin gene on the other chromosome. It is characterized by profound microcytosis with hypochromia but mild or minimal anemia.

β Thalassemia Minor (Thalassemia Trait)

Inheritance of a single beta thalassemia allele results in a mild hypochromic microcytic anemia. Individuals are asymptomatic with mild or absent anemia.[24] The hemoglobin level averages 1 or 2g/dL lower than that seen in normal persons of the same age and sex. Elevations in Hb A_2 and Hb F occur during the early years of life. Hb F levels decline more slowly than normal; the diagnostic elevated Hb A_2 levels are established by about 6 months of age. Strong intrafamilial correlations of both Hb A_2 and mean corpuscular volume (MCV) are noted.[26] Osmotic fragility is decreased due to the presence of target cells that resist hemolysis.[27] The red cell count is increased or normal. The red cells are characteristically hypochromic (mean corpuscular hemoglobin [MCH] <26-27pg) and microcytic (MCV <75-77 fl). The smear shows varying number of target cells, poikilocytes, ovalocytes and basophilic stippling. The reticulocyte count is normal or slightly elevated. Red cell survival is normal, iron utilization is decreased, and slight ineffective erythropoiesis is present. Most patients are asymptomatic.

During pregnancy the anemia of thalassemia trait often becomes more severe, and transfusions are sometimes necessary.[24] Microcytic anemia refractory to iron therapy should always suggest the possibility of thalassemia trait. . In many instances, iron deficiency anemia is erroneously diagnosed and iron therapy is given without significant improvement.

Although a variety of indices calculated from blood count parameters have been suggested to differentiate thalassemia from iron deficiency, each has some degree of inaccuracy; most are no better than the MCV alone. In general, the MCV is rarely >75fl and hematocrit <30 in beta thalassemia trait. In iron deficiency, the hematocrit usually falls to <30 before the MCV falls to <80fl. Free erythrocyte porphyrin levels are normal in thalassemia trait but are elevated in iron deficiency. Direct tests for iron deficiency are preferable for correct diagnosis.

The diagnosis of beta-thalassemia trait is established in most instances by the demonstration of altered proportions of Hb A_2. The level of Hb A_2 in beta- thalassemia trait averages 5.1% (range 3.5-7.0%), approximately twice the normal level (1.5-3.5%); the

Hb A_2/HbA_1 ratio is 1:20 instead of the normal 1:40. This increase is probably due to a post-translational (assembly) phenomenon with increased opportunity for alpha-chains to combine with alpha-chains in the face of beta-chain deficiency. If concomitant severe iron-deficiency anemia occurs, Hb A_2 levels may fall, sometimes into the normal range.[28]

Two mechanisms are proposed in pathogenesis of anemia in thalassemias.The deficit in HbA synthesis produces under-hemoglobinised hypochromic microcytic red cells.Another important factor is diminished red cell survival because of cell membrane damage due to insoluble inclusions of free alpha chains. The inclusion bearing erythroid precursors are prone to intramedullary death and escaping red cells are killed by splenic sequestration and destruction.

Alpha-thalassemia is caused by deficient synthesis of alpha-chain which is encoded by pair of genes on chromosome16.
Reduced synthesis of alpha chain is due to deletions of alpha-globin genes.

In newborn with αthalassemia excess unimpaired γglobin chains forms γ_4 known as Hb-Barts.In adults,excess β-globin chains form HbH,which precipitates in oxidized form in older red cells which are then removed by splenic macrophages.

In sideroblastic anemias,there is decreased production of protoporphyrin or impaired incorporation of iron into protoporphyrin in the erythroid cells.This results in insufficient heme generation and excess iron accumulation in the marrow.

Patients have excess body iron and serum ferritin and hypochromic microcytes in the peripheral smear .For confirmation bone marrow examination is needed which will show ringed sideroblasts.

APPROACH TO MICROCYTIC ANEMIA

In children common causes of microcytic hypochromic anemia are Iron deficiency anemia,Thalassemia and rarely anemia of chronic disease.

Lead poisoning, sideroblastic anemias cause microcytosis but are rare in children.Iron deficiency anemia is most common cause of Microcytic hypochromic anemia.[33,34].

Reticulocyte count is decreased in Iron deficiency anemia and Anemia of chronic disease and it is increased in α or β thalassemia or HbE disease.

RBC count is elevated in (>5 million/cumm) in children with Thalassemia trait and depressed in children with Iron deficiency anemia..

In Thalassemia minor cases the microcytosis is severe than might be expected for the degree of anemia.

Target cells and basophilic stippling tend to be more prominent in thalassemia than Iron deficiency anemia..

Modification of mentzer index can be used to differentiate Iron deficiency anemia and Thalassemia Trait[35] which is based on MCV and RBC count.

MCV/RBC count >14 –suggestive of Iron Deficiency anemia

MCV/RBC count<12- suggestive of Thalassemia trait disorders.

Red cell distribution width (RDW) is a useful parameter to differentiate Iron deficiency anemia from other causes.RDW measures the anisocytosis derived from erythrocyte volume distribution.[36,37,38]

Anisocytosis is an early and prominent finding in IDA and is the first parameter to rise even before appearance of anemia.

An increased Red cell distribution width(RDW) appears to be 90-100% sensitive for Iron deficiency and 50-70% specific.[39]

Increase in red cell distribution width correlates with severity of Iron Deficiency anemia.

In a study mean RDW value was 16.6±1.78, 17.95±1.91, and 20.55±1.32 among mild,moderate and severe anemic children.[40]

Homozygous hemoglobinopathies HbE and HbC tend to be microcytic and normochromic and many target cells are apparent in blood smear.[41,42,]

Homozygous β thalassemia cases show extreme anisopoikilocytosis and nucleated rbcs and target cells in peripheral smear apart from signs of hemolysis and ineffective erythrpoeisis.

A therapeutic trial of oral iron is an appropriate initial diagnostic test for microcytic anemias with features suggestive of Iron Deficiency anemia in Peripheral smear and automated hemogram.[31]

Further investigation is necessary only if there is no response to treatment.

A dose of 6mg/kg/day of elemental iron given in the form of oral ferrous sulfate is the preferred method.[31,]

Reticulocyte count should increase in 5-10 days and the serum Hb should increase by 1gm/dl/week thereafter.

Poor response is due to factors like poor compliance,poor absorption ,incorrect diagnosis and ongoing blood loss.

Further lab analysis is needed in children who are not responding to rule out other causes.

Tests for screening Iron deficiency Anemia are Serum iron,TIBC,serum ferritin and free erythrocyte protoporphyrin.

Serum Iron levels are measurement of Iron bound to transferrin.

Total iron binding capacity (TIBC) is an indirect measurement of transferring in terms of amount of iron it will bind.

Serum Iron has considerable fluctuation in values [43,44,45,46] of individual upto 10-40% within a single day or from day to day.[47,48]

TIBC values show only slight variation.

Normal serum iron-70-200 µgm/dl.

TIBC value-250-435µg/dl.

Transferrin saturation is 20-45% normally.

Values below 16% are noted in Iron Deficiency and Anemia of chronic disease.TIBC is often increased in Iron Deficiency and decreased in Anemia of chronic disease.[49]

Determination of serum ferritin often the only test needed to diagnose Iron Deficiency anemia.Serum ferritin concentrations is proportional to total body iron stores and are not influenced by recent iron therapy.[50]

Ferritin values-10-500 µg/l Sr ferritin is low in Iron Deficiency anemia,(10µgm/L) ,normal in thalassemia and high in Anemia of chronic disease.[51]Sr ferritin can be elevated in infection,inflammation or malignancy as it is an acute phase reactant.[52]

Free Erthrocyte protoporphyrin(FEP) is accumulated in RBCs when iron is unavailable or unable to combine with protoporphyrin to form heme.It is useful in differentiating Iron deficiency anemia(Elevated FEP) from thalassemia minor(normal FEP)[60]

Evaluation of bonemarrow iron stores is used much less nowadays.

In Bone marrow aspirates ,hemosiderin appears as blue granules in Prussian blue staining.[61.]

Normal marrow iron is graded 1+ to 3+.In IDA,marrow hemosiderin is absent .in ACD,iron is present with grade2-3.Iron stores are greatly increased in thalassemia major(5+--6+).Hb electrophoresis should be obtained in patients with microcytosis and target cells with normal serum ferritin levels .

B-thalassemia major children have elevated HbF levels ranging from10-90% and HbA2 levels may be normal or elevated, (5-7%)Hb A2 is elevated in heterozygous carriers of βThalassemia varying from 3.5-7%.[62.]Thalassemia trait causes microcytosis with mild anemia and Hb electrophoresis reveal HbBarts(γ4 tetramers) in new born period.

In HbH disease electrophoresis shows HbH-25%,Hb Barts and low normal levels of HbA2.

HEMOGLOBIN A_2

Measurement of Hemoglobin A_2 (confirmatory test for beta thalassemia trait) is expensive and time consuming.

Raised hemoglobin A_2 level is the gold standard for diagnosis of thalassemia trait. HbA_2 levels can be measured by various methods such as microcolumn chromatography, high performance liquid chromatography (HPLC) and capillary isoelectrofocusing. Subjects with HbA_2 levels of 3.5 per cent and above are considered to have thalassemic trait.

Hb A_2 levels measured should be interpreted as given in the table below. [30]

INTERPRETATION OF Hb A_2 RESULTS

HbA_2 range (%)	Interpretation
> 7.0%	Hb A_2 values of >7.0 % are extremely rare. Exclude a structural variant. Repeat Hb A_2 estimation. Rare beta thalassemia mutations
3.8-7.0%	Beta thalassemia trait Unstable hemoglobin
3.4-3.7%	Severe iron deficiency in Beta Thalassemia trait. Additional delta-chain variant with beta thalassemia trait

	Interaction of alpha$^{(-)}$ and beta thalassemia Rare Beta thalassemia mutations Presence of Hb S, making accurate measurement difficult Interaction of alpha thalassemia and Hb S. Analytical error (repeat analysis).
2.0-3.3%	Normal. δ-β thalassemia (if Hb F elevated). Rare cases of β thalassemia trait, including co-existing β $^{(-)}$ and δ thalassemia and coexisting β and α thalassemia. α thalassemia
<2.0%	δ-beta thalassemia (if Hb F elevated) α thalassemia trait Hb H disease. Additional δ- chain variant present

AIMS & OBJECTIVES

To study the clinical profile of hypochromic microcytic anemia in children aged 2 months to 12 years attending the OPD at Institute of Child Health & Research Centre, Government Rajaji Hospital, Madurai.

To evaluate the role of therapeutic oral iron therapy as an diagnostic approach to hypochromic microcytic anemia

MATERIALS AND METHODS

Study population:

Children attending inpatient and outpatient services at Institute of Child Health and Research Centre, Government Rajaji Hospital, Madurai.

Study group:

A total number of 350 cases of anemia were studied from both inpatient and outpatient services in the age group 2 months to 12 years in the Institute of Child Health and Research Centre, Government Rajaji Hospital, Madurai

Study period:

January 2008 to June 2009

Study design:

Prospective interventional study

Collaborating department:

Department of pathology, Government Rajaji Hospital, Madurai.

Inclusion criteria:

The criteria used in the selection of children was those whose hemoglobin less than 11 gm% in age group less than 6 years and whose hemoglobin less than 12 gm% in age group 6 to 12 years along with peripheral smear showing hypochromic microcytic anemia.

Exclusion criteria:

This study had excluded the cases, who were already diagnosed elsewhere or who were on treatment prior to hospitalisation or peripheral smear showing macrocytic and normochromic normocytic anemia or the neonates.

Ethics committee approval was obtained

Methodology:

Informed consent was obtained from all parents. All the 350 cases of anemia were studied by taking detailed history and thorough clinical examination with meticulous care and the findings were recorded in the predesigned proforma annexed in the last few pages. Hemoglobin estimation and peripheral smear study were done in all cases.

All children in the study group of more than one year of age and those with less than one year of age with no hepato splenomegaly or frontal bossing were dewormed by giving a single dose of Albendazole and were started on oral Iron therapy.

Albendazole was given in a dose of 400mg for children more than 2 years of age and 200 mg for children less than 2 years of age. Oral iron preparations used in this study was Ferrous sulphate. Each ferrous sulphate tablet containing an elemental iron of 20 mg. Iron was prescribed at a dose of 3-6mg/kg/day in 2-3 divided doses. Drugs were given once in 2 weeks. During each visit these children were followed up by Hb estimation and checked for compliance.

At the end of 3 months completion of iron therapy repeat peripheral smear examination was done. Those cases improved with iron that is peripheral smear showing normochromic normocytic type of RBC's were considered as Iron deficiency anemia and they were prescribed iron for one more month to replenish the stores and then iron therapy stopped. Those cases not improved with iron were further evaluated using automated

hemogram, Hb Electrophoresis and bone marrow study as necessary based on individual cases.

All children less than 1 year of age with severe pallor, hepatosplenomegaly and frontal bossing were not given iron therapy. These children were evaluated using automated hemogram and Hb electrophoresis. Bone marrow study was done if necessary.

1. Hb Estimation :

Hb is estimated by using Sahli's hemoglobinometer. It is based on the separation of globin from haemoglobin by treatment with hydrochloric acid to produce acid hematin which is measured by calorimetry.

0.1N Hcl is placed in a graduated tube (Wintrobe's) up to 20^{th} mark. Blood is withdrawn upto 20^{th} mark (20ml) in Sahli's pipette and added to the tube; mixture is allowed to stand for 10 min for complete conversion of acid hematin. The solution is diluted with a few drops of distilled water of a time until colour change matches the standard. The height of solution corresponds to the Hb content.

2) Peripheral blood smear :

Blood smears were prepared on glass slides by wedge method. A drop of capillary blood is placed in the middle of the slide about 1 to 2 cm from one end. A second spreading slide is placed at 30 to 45 angle and moved forward. Smears are dried and stained by Leishman's stain containing Eosin as acidic stain and the methylene blue as the basic stain.

Pour 8-12 drops of Leishman's stain over the slide and wait for two minutes. Then add double the volume of buffer water and wait for 8-10 minutes. Then the wash the slide in running water for 2-3 seconds. Wipe off the excess stain from under surface of the slide and air dry smear. Then smear is examined under the microscope.

3. Hemoglobin Electrophoresis :

In this study, high pressure liquid chromatography was used. 3ml of blood added with EDTA solution was used. With HPLC the Hb fractions are separated based on their ionic interactions with the cationic column under high pressure and by elution with two phosphate buffers differencing in pH and ionic strength. The result is a chromotagram with a percentage of each Hb fraction.

4. Automated blood cell counter :

2 ml of blood was collected by Venepuncture into bottles containing EDTA solution and transported to laboratory.

In this study, COULTER Ac T. DIFF TM Analysis SUN Diagnostics type of cell counts was used. It uses the principle of electrical impendence. It measures Hb, MCV, MCHC, RDW, RBC count, WBC count and platelet count.

5. Bone marrow Aspiration cytology :

Bone marrow is a semifluid and easily aspirated through a 18 gauge size bone marrow needle. It is usually taken from posterior iliac crest or upper end of tibia. Smears are stained with Leishman's stain and assessed for cellularity, Maturation patterns of erythroid and myeloid series and the presence of megakaryocytes.

Statistical Analysis :

The information collected regarding all the selected cases were recorded in a master chart. Data analysis was done with the help of computer using Epidemiological Information Package (EPI 2008).

Using this software frequencies, percentage, mean, standard deviation, chi square and ‘p’values are calculated. Kruskul Wallis chisquare test was used to test the significance of differences between quantitative variables and Yate’s test for qualitative variables. A ‘p’ value less than 0.05 is taken to denote significant relationship.

OBSERVATION, ANALYSIS AND RESULTS

In this study a total of 400 children were included. 350 cases turned up for regular follow up. 50 cases lost the follow up.

Table – 1

Incidence of Anemia in relation to Sex

Sex	Cases	
	No.	%
Male	205	58.6
Female	145	41.4
Total	350	100

Out of 350 children in the study group, 205 (58.6%) were males and 145 (41.4%) were females.

Table – 2

Severity of Anemia

S.No.	Degree of anemia	No.	%
1.	Severe (<7 mg%)	142	40.6
2	Moderate (7-9.9gm%)	188	53.7
3	Mild (10-10.9gm%)	20	5.7

Of the above analysis, 142 cases of severe anemia, 188 cases of moderate anemia and 20 cases of mild anemia were documented. Since this is a hospital based study number of moderate to severe anemia cases are more.

Table – 3

Relationship between Sex and Severity of Anemia

Degree of anemia	Sex		
	Male	Female	Total
Mild (10 – 10.9 mg%)	9	11	20
Moderate (7.9 – 9 mg%)	114	74	188
Severe (< 7 mg%)	82	60	142
Total	205	145	350

'p' = 0.5364 (not significant). There is no statistically significant difference in the relationship between sex and severity of anemia.

Table – 4

Age Distribution

Age group	No.	%
Less than 1 year	15	4.3
1 – 3 yrs	126	36
3.1 – 6 yrs	106	30.3
6.1 – 12 yrs	103	29.4
Total	350	100
Range	Months – 12 years	
Mean	4.4 years	
SD	3.2 yrs	

Of the above analysis, 15 cases were less than 1 year of age, 126 case were between 1-3 year of age, 106 cases were between 3-6 years of age, 103 cases were between 6-12 years of age.

The mean age of this study is 4.4 years.

Table – 5

Complaints

Complaints	No.	%
Easy fatiguability	339	96.9
Pica	66	18.9
Passing worms in stools	9	2.6
Fever	340	97.1
Breathlessness	21	3.1

The various modes of presentation of anemia were as follow. 331 cases had easy fatiguability. 66 cases had H/o pica, 9 cases had H/o passage of worms in stools, 340 cases had fever and 11 cases presented with breathelessness. Many cases had more than one complaints.

Table – 6

Signs

Signs	No.	%
Pallor	350	100
Koilonychia	47	13.4
Frontal bossing	10	2.9
Pedal edema	1	0.3
Hepatomegaly	69	19.7
Splenomegaly	68	19.4

In this study, the various findings among children with anemia were as follows. Pallor was present in almost all cases. Koilonychia in 47 cases, frontal bossing in 10 cases. Pedal edema in 1 case. Hepatomegaly in 69 cases and splenomegaly in 68 cases. Many cases had more than one signs.

Table – 7

Splenomegaly and type of Anemia

Type of Anemia	Splenomegaly		
	Mild < 4 cm	Moderate 4 to 7 cm	Massive > 7 cm
IDA	51	0	0
Others	5	10	2

In this study, splenomegaly was present in 68 cases (19.4%) Out of 68 cases, 51 cases (14.8%) of IDA had mild splenomegaly. Remaining 17 cases belongs to Thalassemia group. Among them 5 cases had mild splenomegaly. 10 cases had moderate splenomegaly and 2 case had massive splenomegaly.

Table – 8

Hb %

Hb%	Range	Mean	SD
Initial Hb	3.2 - 10.5	7.36	1.31
Repeat Hb	5.4 – 12.8	11.8	0.79
Increase in Hb	0 – 8	4.4	1.4
% of increase	0 – 250	64.5	32.3

From the above analysis, the mean Hb% at the time of entry to study was 7.36gms%. The mean Hb% after the iron therapy was 11.8 gms%. The mean increase in Hb was 4.4 gms%.

Table – 9

RBC Count

	RBC count
Range	1.45 – 4.33
Mean	2.9
SD	0.61

In this study, the average RBC count was 2.9 million cells / cumm.

Table – 10

Response to Iron

Response to Iron	Cases	
	No.	%
Positive	333	95.1
Negative	7	2
Iron not given	10	2.9%
Total	350	100

In this study, 333 cases improved with oral iron therapy, 7 cases not improved with iron and 10 cases does not received iron therapy.

Table – 11

Diagnosis

Diagnosis	Cases	
	No.	%
Iron deficiency anemic	333	95.1
Others - total	**17**	**4.9**
Alpha Thalassemia	2	0.6
Thalassemia intermedia	1	0.3
Thalassemia major	7	2.0
Thalassemia trait	5	1.4
Hb variant E	1	0.3
Unstable Haemoglobinopathy	1	0.3
Total	350	100

From the above analysis, Iron deficiency anemia is the major cause which constitutes about 95% (333cases). The other causes were 7 cases of Thalassemia major, 5 case of Thalassemia trait; thalassemia intermedia one case; Hb variant E one case; unstable hemoglobinopathy One case and alpha thalasemia two cases.

B : Relationship between Type of anaemia and other variables

Table – 12

Age and type of anemia

Type of Anemia	Age in years	
	Mean	SD
I DA	4.53	3.22
Others	2.69	2.26
P	0.0107 Significant	

From the above analysis the mean age of IDA was 4.58 yrs and in remaining other causes the mean age was 2.69 with a 'p' value of 0.0107

Thus the age of presentation of different type of anemia is statistically significant.

Table – 13

Type of Anemia

Sex	Type of Anemia			
	IDA		Others	
	No.	%	No	%
Male	194	94.6	11	5.4
Female	139	95.9	6	4.1
'p'	0. 7841 Not significant			

Among the 205 males, 194 were IDA and 11 were other types. Among the 145 females, 139 were IDA and 6 were other types.

P – 0.07841. The relationship between the type of anemia and sex are not statistically significant.

Table – 14

Type of Anaemia and Hb% changes

Hb	Type of Anaemia				'p'
	IDA		Others		
	Mean	SD	Mean	SD	
Initial Hb	7.42	1.29	6.12	1.12	0.0001 significant
Repeat Hb	11.89	0.41	7.7	2.29	0.001 significant
Increase	4.46	1.34	1.81	1.97	0.0012 significant
% of increase	65.2	31.9	30.6	34	0.0028 significant

From the above analysis, in case of IDA, the mean rise in Hb % after oral iron therapy was 4.46gm% whereas in the other types the mean rise in Hb% was only 1.81%

Thus the type of anemia and Hb% changes after oral iron therapy were statistically significant.

C : Relationship between Response to Iron and other factors

Table – 15

Age and response to iron

Response to Iron	Age in years	
	Mean	SD
Positive	4.49	3.23
Negative	4.57	2.21
P	0.5331 Not significant	

From the above analysis, the mean age of the children responded to oral iron was 4.49 years and those not responded was 4.57 years.

P – 0.5339.

Thus the age group and response to iron is not statistically significant.

Table – 16

Type of Anemia and Response to Iron

Type of Anemia	Response to Iron					
	Positive		Negative		Not given	
	No.	%	No.	%	No.	%
IDA	333	100	0	0	0	0
Others (17)	0	0	7	41.2	10	10
'p'	0.0001 significant					

From the above analysis, it is seen that all cases of IDA, 333 cases improved with iron and among the other types of anemia 7 cases not improved and 10 cases were not started on iron therapy.

P - 0.0001

Thus the relation between type of anemia and responds to iron is statistically significant.

Table – 17

Sex and Response to Iron

Hb	Response to Iron			
	Positive		Negative	
	No.	%	No.	SD
Male	196	97.5	5	2.5
Female	140	98.6	2	1.4
'p'	0.3883 Not significant			

From the above analysis, 196 males improved with iron and 5 cases not improved. Among the females 140 cases improved with iron and 2 cases not improved.

P = 0. 3883

There is no statistically significant relation between sex and response to iron.

Table – 18

Response to Iron and Hb% changes

Parameters	Response to Iron				'p'
	Positive		Negative		
	Mean	SD	Mean	SD	
Initial Hb	7.4	1.3	6.68	0.87	0.118 not significant
Repeat Hb	11.87	0.53	7.75	1.16	0.0006 significant
Increase	4.45	1.35	1.45	0.21	0.0012 significant
% of increase	65.15	32	23.12	1.91	0.003 significant

From the above analysis, the mean rise in Hb% in cases improved with iron was 4.45gm% whereas only 1.45%, in cases with no response to iron therapy.

Thus the response to iron and rise in Hb% are statistically significant.

DISCUSSION

The clinical profile of hypochromic microcytic anemia such as age and sex distribution, the presenting complaints, clinical findings, degree of anemia, various etiological factors, importance of oral iron therapy as a diagnostic test, using higher investigations only when necessary were analysed and discussed here.

Sex :

In this study, the incidence of hypochromic microcytic anemia is more common in males (58.6%) than female children (41.4%). But this is not statistically significant. This is in accordance with the fact that sex plays important role in determining prevalence only in older age group.

Age : In this study, 4.3% are less than one year of age. 36% are between 1- 3 years of age. 30.3% are between 3 -6 years of age and 29.41 are between 6-12 years of age.

Thus the most common age group affected is in between 1-3 years of age.

This is similar to Omprakash et al study which states that the peak incidence of nutritional anemia in children occurs between 6 months to 3 years of age.

Presenting Complaints :

In this study, the presenting complaints of hypochromic microcytic anemia includes easy fatiguability (96.9%), Pica in 18.9%, worm infestation in 2.6%, fever in 97.1% and breathlessness in 3.1%.

Fever is the most common presenting complaints. Since this is a hospital based study, most of the cases who came for upper respiratory tract infection and viral fever are incidentally found to be anemic and included in the study. Hence fever is present in majority of cases.

Out of 350 cases, 339 cases have the compliant of easy fatiguability. This confirms the observation in western literature that easy fatiguability is the most common symptoms in anemia particularly Iron deficiency anemia.

In this study PICA is present in 66 cases (18.9%). This symptom is found in 50% children in a study conducted by Crosby et al.

Worm infestation is present only in 2.6% of cases. The low prevalence of parasitism in this study may be because of wide spread use of antihelminithic drugs by health officials.

Signs :

In this study, Pallor is present in all cases. Out of 350 cases, 47 cases (13.4%) have koilonychias. 2.9% of cases have frontal bossing. Malar prominence along with frontal bossing are even present in severe iron deficiency anemia

Hepatomegaly is present in 69 cases (19.7%) and splenomegaly is present in 68 cases (19.4%) .In this study hepatosplenomegaly is seen in 14.8% of the cases of IDA. Mild degree of hepato splenomegaly is present in iron deficiency anemia.

Piyush Gupta and OPGhai states that Spleen is enlarged in 15% of cases of IDA.

OmPrakash et al states that mild degree of hepato splenomegaly is not uncommon in IDA.

In this study, the mean initial Hb% is 7.36gm%. The mean Hb% after oral iron therapy is 11.8gms%

Iron deficiency anemia is the most common cause of Nutritional anemia world wide. It is the most common type of hypochromic microcytia anemia.

In country like India, Iron deficiency anemia co exists with almost every type of others anemia in children. Hence it has to be corrected to come for final diagnosis.

In this study, all cases of hypochromic microcytic anemia are given Oral iron in the form of ferrous sulphate tablets containing 20 mg elemental iron at a dose of 3-6 mg / kg / day in 2-3 divided doses.

Oral iron therapy is given for 3 months. At the end of 3 months, cases improved with iron are those with true iron deficiency anemias. Only those cases not improved with iron therapy are further investigated.

The above approach is supported by Hermiston et al, the pediatric clinics of North America. It states that “In hypochromic microcytic anemia, therapeutic trial of oral iron is an appropriate initial diagnostic test. Further investigation is unnecessary unless there is no response. A dose of 6 mg / kg / day of elemental iron

divide bid to tid is indicated. Ferrous sulphate is the most bioavailable preparation.

Thalassemia is the next most common cause of hypochromic microcytic anemia. It is an inherited genetic disorder causing real burden to the family and the society. Thalassemia trait cases have to be detected to prevent future homogenous thalassemia major cases. Hb electrophoresis is the investigation of choice. If there is coexisted iron deficiency in thalaseemia trait case, even by Hb electrophoresis it cannot be detected.It has to be corrected prior to Hb electrophoresis.

This emphasizes once again that oral iron has to be given to all cases of hypochromic microcytic anemia as done in this study.

This concept is supported by Hermiston et al. The pediatrics clinics of North America that the patient should not be iron deficient at the time of electrophoresis as iron deficiency depresses delta globin synthesis, obscuring a rise in HbA2.

Wintrobe's Hematology states that the diagnosis of heterozygous beta thalassemia rests on the demonstration of an increase in HbA2 or HbF is an individual whose peripheral blood

smear shows microcytosis, hypochromia, target cells and basophilic stippling.

Iron deficiency causes reduced levels of HbA2. Elevated levels cannot be demonstrated until the iron deficiency is corrected.

Nelson states that thalassemia trait is frequently misdiagnosed as iron deficiency in children. A short course of iron and reevaluation is all that is required to identify children who will need further evaluation

In this study, out of 350 cases, 333 cases are Iron deficiency anemia (95.1%)

The most common cause of hypochromic microcytic anemia in this study is Iron deficiency anemia. This finding is similar to the studies conducted by Viswanath D et al where the incidence is 89%.

Another study by Fazal Razir Khan et al states that the incidene is 92% and by Kapoor D et al it is 88%.

As per ICMR study (VP Choundhry et al in 1995) on anemia. Iron deficiency was proved to be the commonest cause.

Out of 350 cases, 142 cases (40.6%) were severe anemia, 188 cases (53.7%) were moderate anemia and 20 cases (5.7%) were mild anemia. Since this is a hospital based study. Moderate to severe anaemia are more than the mild anemia cases.

Out of 350 cases, 7 cases did not improve with oral iron therapy. These cases were further evaluated by automated hemogram and Hb electrophoresis.

Automated hemogram was done in all these 7 cases to demonstrate low MCV (less than 75fl) even after 3 months iron therapy. Automated hemogram was done to confirm the findings on peripheral smear.

Out of 7 cases, 5 cases of thalassemia trait (1.4%) was detected by Hb electrophoresis. All the 5 cases showed elevated HbA2 .

One case of Thalassemia intermedia was detected. He is an 8 year old boy presented with pallor, mild hepatosplenomegaly and did not improve with 3 months of oral iron therapy. Hb electrophoresis showed raised HbF, raised HbA2 and low HbA.

The remaining one case was Hb variant E. She is a 7 year old girl presented with pallor, liver enlarged 2cm below right costal margin, and spleen enlarged 4cm below left costal margin, did not improve with oral iron. Hb electrophoresis showed low HbA and raised HbE, normal HbA2.

In this study, out of 350 cases, 10 cases have not received iron. These children typically presented with severe pallor, hepatosplenomegaly and many were in the age group of less than one year. Hb electrophoresis was done for these cases without prior oral iron therapy.

Among the 10 cases, 7 cases are diagnosed as Thalassemia major. It showed elevated HbF and low HbA.

One case of unstable hemoglobinopathy was diagnosed. He is a 1 year old boy presented with severe pallor, fever and hepatosplenomegaly. Peripheral smear showed severe hypochromic microcytic anemia. Hb electrophoresis showed decreased HbA, Normal HbA2, HbF not elevated and unidentified Peak was present. Child became more pallor and sick during intercurrent

illness and was symptomatically treated. In between the illness the boy was normal without pallor.

Of the remaining 2 cases, one was that of a 4 months old female child and another that of a 7 months old female child both of whom presented with severe pallor and hepato splenomegaly. Peripheral smear showed Severe hypochromic microcytic anemia.. Hb electrophoresis were normal in both cases. Both were subjected to bone marrow smear study. In both cases erythroid hyperplasia were seen. These 2 cases could be that of Alpha thalassemia. Hb electrophoresis by HPLC method could not detect alpha thalassemia. Genetic studies are further required to confirm the diagnosis.

In this study, the incidence of Thalassemia (including Thalassemia major, intermedia and trait) is 4%.

World wide, the frequency of thalassemia varies from 3-17% in different population. In a study by Salma Shaikh et al the incidence of thalassemia is 11%.

CONCLUSION

1. The most common cause of hypochromic microcytic anemia in children is Iron deficiency anemia (nutritional anemia)
2. Thalassemia group is the next common cause
3. Children in 1-3 years of age are commonly affected
4. Fever and easy fatiguability are the major presenting features.
5. Mild hepatosplenomegaly and frontal bossing can occur in Iron deficiency anemia.
6. A therapeutic trial of oral iron is an appropriate initial step in the diagnostic approach of hypochromic microcytic anemia.
7. Costly investigations like Hb electrophoresis and bone marrow study are required only in selected cases.

LIMITATIONS

- Though measures were taken to increase the follow up of cases with hypochromic microcytic anemia the dropout was 12.5 %
- Causes of hypochromic microcytic anemia such as sideroblastic anemia and lead poisoning are not made out.

RECOMMENDATIONS

Cases with hypochromic microcytic anemia should be given a trial of therapeutic oral iron therapy before subjecting them to Hb electrophoresis as iron deficiency depresses delta globin synthesis, obscuring a rise in HbA2.

BIBLIOGRAPHY

1) Stohlman F.Kinetics of erythropoeisis;In;Gordon AS;regulation of hematopoeisis.Vol 1,New york;Appleton-century-crofts;1970;317

2) OrlicD Ultrastructural analysis of erythropoeisis;In Gordon AS,ed,Regulation of hematopoeisis Vol 1,New york;Appleton-century-crofts;1970;271

3) Tooze J.davies HG;The occurrence and possible significance of Hb in the chromosomal regions of mature erythrocyte nuclei.J cell Biol 1963;16;501-511

4) WHO (1968) techn .Rep.Ser.No.405

5) Soed.S.K. and U.russia (1986);Ann of Nat Acad of Med.Sci.India 22(4) 235.

6) Sahibzada Syed Masoodus Syed, Muhammad Saeed Razi, Saleh Muhammad, Sohail Amjad, Ahmed Affifi. Frequency of Anemia in Pediatric out patient departmentPak Paed J Mar 2004;28(1):35-6.

7) William's Hematology 7th edition ;page 413

8) Martin PL et al.The anemias Philadelphia;Lippincott 1994:165

9) Verma et al;Indian Paediatrics 1998 Dec 35(12);1181-6

10) Indian journal of medical sciemces Pub date 01/02/08Author-sinha N;Deshmukh p;gary B

11) Kapoor D.Agarwal KN,Iron status of children aged 9-36 months ;ICDS project Indian Pediatrics 2002 Feb 136-44

12) Padmanabhan A et al ;Ann trop Paediatrics -2001 Mar21/17;45-9High prevalence of microcytic anemia in omani children –a prospective study.

13) J.Trop Pediatr 1999 Aug ;45(4);221-5 ;the pattern of common anemia among saudi children.

14) Molla et al JDMA -1992 may 42(5);118-121

15) Azmat Manzoor, Muhammed Tayyib, Tahira Tasnim. Anemia in school childrenPak Postgrad Med J Mar 2003;14(1):44-7.Department of Pathology, Postgraduate Medical Institute, Lahore.

16) Aulukh R.Sohi I.Singh T, Kakkar N Indian J Pediatr ;2009 ;Mar 76(3);265-8

17) Dr.Arvind Lal et al-The Thalassemia saga express health care

18) Lokeshwar M.R,Shah N .Thalassemia . I A P Textbook of Pediatrics;2nd edition:page 622-629

19) Keith Quirolo and Elliott Vichinsky.Nelsons text book of pediatrics .17th Edition Chapter 454;page1630-1634

20) El sahn F,Salam S.East Mediterr Health J 2000;6;1017-25

21) Venter A,Vender Pol J.L East Mediterr Health J 1995; 64-70

22) Gordon .N.Iron Deficiency and the intellect bruin dev 2003;25;3-8

23) Schwartz;E;IDA; nelson textbook of pediatrics 16thedition 1469-71

24) Elias Schwartz ,Edward j.Thalassemia Syndrome.Hematology basic principles and practice. 2nd edition.Chapter 42, page no 586 to 608

25) Nancy F,Oliver M.D.The β-thalassemias.The New England Journal Of Medicine 1999:vol 341.number 2:99-109

26) Berman BW et al. Hematology of beta thalassemia trait. Age related development aspects and intrafamilial correlation.J Ped 97:901,1980

27) Sears DA et al. Red cell osmotic fragility studies in hemoglobin C-beta thalassemia: osmotically resistant microspherocytes. Clin Lab Hematol.2003;25(6):367-72

28) Wasi Pet al ;The effect of iron deficiency on the levels of Hb A2 and E.J Lab Clin Med .71:85,1968

29) The evaluation of various mathematical RBC indices and their efficacy in discriminating between Thalassemic and Non-Thalassemic microcytosis. AJCP, 1996; 106: 201-205

30) Dacie N,Levis SM 1991. Practical-Hematology, 7th edition, London: Churchill livingstone

31) M.L.Hermiston W.C. Mentzer Pediatr Clin N Am 49(2002

32) Baar.H.,Die Anamien: In die klinische, haematological des klindesalter.Leipzig,Franz deuticke 1928

33) Olivares M.et al.Anemia and iron deficiency disease in children .br med B cell 1999;55;334-43.

34) Goodnough LT,Skikie B,Brugnan C,Erythropoeitin,iron, and erythropoeisis, Blood 2000;96;823-33.

35) Mentzer WC .Jr .Differentiation of Iron deficiency from Thalassemia Trait Lancet 1973;882

36) England Jm.ward SM,Doun MC.Microcytosis,anisocytosis and the red cell indices in Iron deficiency.Br j Hematol 1976;34(4)589-597.

37) Bessman JD,Feinstein D. Quantitative anisocytosis as a discriminant between iron deficiency and thalasemia minor.

38) Johnson cs,Teges C. Beutler E. thalassemia minor Routine erythrocyte measurements and differentiation from Iron deficiency .Am J Clin pathol 1983 80(1) 31-36

39) McClure S.Custer E.Bossman JD.Improved detection of early iron deficiency in non-anemic subjects .JAMA 1985;253(7)1021-1023

40) Aulokh R.sohi I.Singh T.kakkar N RDW in diagnosis of ID with MHA Indian J pediatr 2009 Mar 76(3) ;265-8

41) Cunningham TM,Hemoglobin E in Indochinese refugees .West J Med 1982;137(3)186-190

42) Fairbanks VF.et al. Homozygous HbE mimics β thalassemia minor without anemia(or) hemolysis,am J hematol 1980;8(1) 109-115

43) Marsh WL Jr.Koenig HM.,The lab evaluation of microcytic red cells Crit rev Clin lab Sci 1982;16(3) 195-254

44) Dallmann PR,Diagnosis of anemia and iron deficiency ;Analtic and biologic variations of laboratory tests .Am J Clin Nutr 1984;39(6)937-941

45) Statland BE,Wikel P.Relationship of day-to- day variation in serum Iron conc Am J Clin Pathol 1977;67(1) ;84-90.

46) Itano M.CAP comprehensive chemistry Serum iron survey Am J Clin Pathol 1978;70;516-522

47) Statland B,Winkel P.Bokland H Variation of serum Iron conc in healthy men,clin Biochem 1976;26-29

48) Werkman HP et al The short term .Iron rhythm ;Clin Chem Acta 1974;53;(11);65-68

49) Wiana FH Jr et al.Discrimination between ID and ACD Am J Clin Pathol 2001;15(1)112-118

50) Worwood M. Sr Ferritin CRC Crit Rev Clin labSci.1979;10(2); 171-204

51) Lipshitz ,Cook JD et al. Sr ferritin as an index of iron stores N Engl J med 1974-290;1213-1216

52) Hulthen L.LinstedtG.Et al, Effect of a mild infection on sr ferritin concentration Eur J clin Nutr 1998-;52;376-9

53) Chauffard M.A, les icterus hemolytique, sem.Med.28:49,1908

54) Chauffard.M.A., pathogene de I ictera congenital del 1 adulte,sem.Med.27:25, 1907

55) Cooley.T.B. and lee P., series of cases of splenomegaly in children with Anemia and peculiar bone changes, American pediatric society,37: 29,1925

56) Dr. V. P. Choudary: Iron deficiency Anemia. Year book – 1995: pediatrics

57) Sarada Sidhu : Incidence of Anemia among scheduled caste pre school children of Punjab. Indian Journal of Maternal and child health,1996, 7(3): 76-77

58) G. C .De Gruchy, clinical hematology in medical practices 5th edition 1989.

59) Frank.A . Oski., Hematology of Infancy and childhood by Nathan and Frank . A. Oski., 5th edition

60) Gansslen . M . Uber., Hamolytischen Ikterus. Dtsch. Arch, Klin med.140:210, 1922

61) Maxwell M. Wintrobe, John N. Lukens and G. Richard tec. The Approach to the patient with Anemias. Wintrobe' s clinical hematology 1993

62) Serden MA et al.The role of EP in IDA in children J trop Pediatr 2000;46-323-6

63) Rath .C.Finch CA .Sternal marrow hemosiderin 1948;3;81-86

64) Weatherall DJ.Clogg JB.The thalassemia syndromes ;Oxford Blackwell science 2001

65) Gomber S, Sanjeev, Madan N. Validity of Nestroft in screening and diagnosis of beta-thalassaemia trait. *Journal of tropical pediatrics*, 1997, 43(6):363–6.

PROFORMA

CASE NO: Height: Weight: Blood Group: Rh: + / -	NAME: AGE: SEX: Male/Female
ADDRESS: PHONE NO:	

FATHERS NAME:	MOTHERS NAME:
Occupation:	Family Income:
community	Consanguinous:
No. of Brothers:	No. of Sisters:

COMPLAINTS	YES	NO	DURATION
Easy fatiguability			
Fever			
Recurrent Cough / Cold			
Shortness of Breath			
Irritability			
Picca			

PAST HISTORY	YES	NO	DURATION
H/o Jaundice			
H/o Blood transfusion			
H/o Drug (Iron) intake			
H/o Sibling death			
H/o Family member taking Blood transfusion			
H/o Passing worms in stools			

Diet Veg / Non. Veg	

CLINICAL FEATURES

GENERAL EXAMINATION: **VITAL SIGNS T RR PR BP**	
PALLOR	**LN ENLARGEMENT**
CLUBBING	**CYANOSIS**
ICTERUS	**PEDAL EDEMA**
FRONTAL BOSSING	**KOILONYCHIA**
RESPIRATORY SYSTEM	**CARDIOVASCULAR SYSTEM:**
TRACHEA	**HEART SOUNDS :**
AIR ENTRY	**MURMURS :**
ADDED SOUNDS	**IF YES DESCRIBE :**

ABDOMEN	CNS
DISTENSION / TENDERNESS	CRANIAL NERVES
ORGANOMEGALY	MOTOR SYSTEM
MASS / FREE FLUID	SENSORY SYSTEM

Investigations

Hb -

Blood group -

Peripheral smear -

Complete Hemogram -

Automated hemogram -

Hb electrophoresis -

Bone marrow study -

LIST OF ABBREVIATIONS

IDA	–	IRON DEFICIENCY ANEMIA
Hb	–	HEMOGLOBIN
MCV	–	MEAN CORPUSCULAR VOLUME
MCHC	–	**MEANCORPUSCULARHEMOGLOBIN CONCENTRATION**
MCH	–	MEAN CORPUSCULAR HEMOGLOBIN
RDW	–	RED CELL DISTRIBUTION WIDTH
RBC	–	RED BLOOD CELL
WBC	–	WHITE BLOOD CELL
EDTA	–	ETHYLENE DIAMINE ACETIC ACID
WHO	–	WORLD HEALTH ORGANISATION
NFHS	–	NATIONAL FAMILY HEALTH SURVEY
HC,MC	–	HYPOCHROMIC MICROCYTIC
NC,NC	–	NORMOCHROMIC NORMOCYTIC
Dl	–	DECILITRE
fl	–	FEMTOLITRE
µg	–	MICROGRAM
PS	–	PERIPHERAL SMEAR
HPLC	–	HIGH PRESSURE LIQUID CHROMATOGRAPHY
Hcl	–	HYDROCHLORIC ACID
SD	–	STANDARD DEVIATION
α	–	ALPHA
β	–	BETA
EC	–	ELONGATED CELLS
TC	–	TARGET CELLS
OPD	–	OUT PATIENT DEPARTMENT
TI	-	THALASSEMIA INTERMEDIA
TT	-	THALASSEMIA TRAIT
TM	-	THALASSEMIA MAJOR
UH	-	UNSTABLE HEMOGLOBINOPATHY
AT	-	ALPHA THALASSEMIA
HBE	-	HEMOGLOBIN VARIANT E
P	-	POSITIVE
N	-	NEGATIVE
M	-	MALE
F	-	FEMALE